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Vascular reactivity in cardiopulmonary bypass

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Chapter 6

Adjunct nitrous oxide normalizes vascular reactivity changes after hemorrhagic shock in mice under isoflurane anesthesia¹

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Abstract

Background: Hemorrhagic shock is associated with changes in vascular responsiveness that may lead to organ dysfunction and ultimately multiple organ dysfunction syndrome. Volatile anesthetics interfere with vasoresponsiveness, which may contribute to organ hypoperfusion. In this study, we examined the influence of adjunct nitrous oxide on the vascular responsiveness after short-term hemorrhagic shock under isoflurane anesthesia.

Methods: Spontaneously breathing mice ($n=31$, $27.6 \pm 0.31\text{g}$) were anesthetized with isoflurane (1.4%) or with isoflurane (1.4%) and adjunct nitrous oxide (66%). Both groups were divided in Sham, Shock and Resuscitated groups. Vascular reactivity to phenylephrine and acetylcholine and expression of cyclooxygenases were studied in aorta.

Results: In isoflurane-anesthetized groups, the contractile response to phenylephrine was increased in Shock as compared with the Sham and Resuscitated groups ($E_{\max}=3.2\pm0.4$, 1.2 ± 0.4 and 2.5 ± 0.5 mN, respectively). Adjunct nitrous oxide increased phenylephrine contraction to a similar level in all three groups. In Sham-isoflurane group, acetylcholine caused a biphasic response: an initial relaxation followed by a contractile response sensitive to cyclooxygenases inhibition by indomethacine. The contractile response was abrogated in isoflurane-anesthetized groups that underwent shock. In all groups, adjunct nitrous oxide preserved the contractile phase. Shock induced a downregulation of cyclooxygenases-1, which was normalized by adjunct nitrous oxide.

Conclusion: Adjunct nitrous oxide attenuates shock-induced changes in vascular reactivity and cyclooxygenases expression of mice under isoflurane anesthesia. This implies that vascular reactive properties during anesthesia in hemorrhagic shock conditions may be influenced by the choice of anesthetics.

Introduction

Changed vascular reactivity in hemorrhagic shock (HS) may represent an important factor in the development of a multiple organ dysfunction syndrome. The mechanisms underlying this syndrome are not fully understood. However, early changes in vascular responsiveness may be of significance, as arterial hypotension and vascular leakage have been recognized as hallmarks of the post-shock state.¹⁻³ Indeed, hypotension has been associated with altered vascular reactivity to different modulators of vascular tone.¹ Several mechanisms have been proposed to cause post-shock vascular hyporeactivity including systemic and local release of nitric oxide, decreased plasma levels of vasopressin and changes in properties of vascular smooth muscle cells.²⁻⁵

Patients with hemorrhagic shock frequently receive anesthesia to facilitate diagnostic and therapeutic procedures. Many of the agents employed interfere with vasoresponsiveness and/or influence the therapeutic effectiveness of vasoactive drugs, and the development and outcome of hemorrhagic shock. Volatile anesthetics may evoke peripheral vasodilatation, myocardial depression, and lower sympathetic nervous system activity, subsequently leading to a decrease in blood pressure.^{6, 7}

Moreover, these agents may influence systemic secretion of vasoconstrictors such as vasopressin, angiotensin, and endothelin, further contributing to a reduction in blood pressure and deterioration of organ microcirculation.^{8,9}

Nitrous oxide, still widely used as an adjunct in various anesthetic techniques, has also been implied to influence hemodynamics. Adjunct nitrous oxide to sevoflurane (at 1.5 Minimum Alveolar Concentration [MAC]) or isoflurane (at 1.45 MAC) stabilizes several hemodynamic parameters of regional and systemic flow.¹⁰⁻¹² The mechanisms through which nitrous oxide offsets the hemodynamic effects of volatile anesthetics are still not fully understood,¹³ although venoconstrictor effects of nitrous oxide and sympathetic stimulation have been proposed as a possible explanation.¹²

The purpose of this study was to determine the change in vascular responsiveness obtained from mice after short-term hemorrhagic shock under isoflurane anesthesia, and to investigate the effects of adjunct nitrous oxide administration on the observed changes.

Materials and Methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Groningen (Groningen, the Netherlands). This study was performed in 31 adult mice (27.6±0.31g; Harlan, Zeist, The Netherlands) housed under standard conditions with free access to food (standard rat chow) and drinking water throughout the study.

Experimental animals were assigned to two groups: A) a group of mice that were anesthetized with isoflurane (ISO, 1.4%) in oxygen/air mixture (O₂:33%), and B) a group of mice that were anesthetized with isoflurane (1.4%) and adjunct nitrous oxide (N₂O, 66%) in oxygen (33%). Each group was divided in three experimental sub-groups (n = 5-6 per group). Sham animals were anesthetized and cannulated but not hemorrhaged, and kept under anesthesia for 90 min before being killed. Shock animals underwent anesthesia and hemorrhage and were sacrificed after 90 min of hemorrhagic shock. Resuscitated animals underwent anesthesia with hemorrhagic shock for 90 minutes, followed by resuscitation with 6% hydroxyethyl starch 130/0.4 (Voluven; Fresenius-Kabi, Bad Homburg, Germany). The resuscitation volume was twice the estimated volume of the blood withdrawn to induce hemorrhagic shock. Resuscitated mice were sacrificed 24 hours after induction of shock. All animals were breathing spontaneously throughout the protocol.

A fixed-blood pressure model of hemorrhagic shock was used as described previously.¹⁴ Briefly, after induction of anesthesia the left femoral artery was cannulated with polyethylene tubing (internal diameter of 0.28 mm and an external diameter of 0.61 mm). Hemorrhagic shock was induced by blood withdrawal until mean arterial pressure (MAP) reached 30 mmHg; this blood pressure reduction was reached in approximately 11 minutes. An initial 0.5 ml of blood was withdrawn followed by additional portions of about 0.1 ml mean arterial pressure reached 30 mmHg. Total amount of blood withdrawn was estimated from the number of portions taken from the animal. Blood was collected in a heparinized 1-mL syringe to prevent clotting. Hypotension at 30 mmHg was maintained during 90 min with a continuous monitoring

of MAP. Small increments of blood were infused or withdrawn during the shock period to maintain MAP at 30 mmHg. In both Shock-groups, blood gas analysis was measured twice: just after femoral cannulation ($t=0$ min) and after the shock period immediately before killing ($t=90$ min). In both Resuscitated groups, blood gas analysis was performed just after femoral cannulation ($t=0$ min) and immediately before killing ($t=24$ hours). In Sham-groups, blood gas analysis was performed only immediately before sacrifice (90 min) to avoid potential activation of endothelium and modification of vascular reactivity by withdrawal of blood at $t=0$. After killing, freshly isolated thoracic aorta was collected for *in vitro* vasomotor studies. Abdominal aorta was removed and snap-frozen in liquid nitrogen without pretreatment with any drug, and stored at -80°C until analysis.

Vasomotor responses

After removal, the descending thoracic aorta was immediately placed into cold physiological saline. Freshly isolated thoracic aortic rings (1.5 - 2 mm in length) were mounted on two 200 μm stainless wires in the individual myograph baths wire myograph (Danish Myo Technology A/S, Aarhus, Denmark), containing 6ml of Kreb's solution, warmed to 37°C and bubbled continuously with 95% O_2 /5% CO_2 to maintain pH at 7.4. Length of aortic strips was assessed by microscopy. Aortic rings were equilibrated for 40 min until they were at a steady baseline. Then, rings were subjected twice to stimulation with potassium chloride (60 mM) in order to obtain reproducible contractile responses. Contraction was measured by obtaining concentration-response curves to phenylephrine (10 nM-100 μM). Endothelium-dependent relaxation was measured by obtaining concentration-response curves to acetylcholine (10 nM-300 μM) in rings precontracted with phenylephrine. The influence of vasoactive prostanoids on the response to acetylcholine was examined by incubating rings with the non-specific cyclooxygenase inhibitor indomethacin (10 μM) administered 20 min before application of phenylephrine. In the end of each experimental protocol, endothelium-independent relaxation was measured by applying the nitric oxide-donor sodium nitroprusside (0.1 mM).

Western blotting

The methods used were described previously.^{15, 16} Briefly, after grinding, the frozen aortas were placed in 300 μl of boiling 2% SDS followed by pounding by a polytron (Kinematica AG Littau, Switzerland). Then, samples were centrifuged (4000 rpm, 1 min) and boiled (95°C) for 5 min. After a second centrifugation (13000 rpm, 3 min), supernatant was collected and used for measurements. Protein concentration was determined by Bio-Rad Protein Assay (Bio-Rad, Hercules, CA). Forty μg of total protein from each sample was separated on 4-20% Precise Protein Gels (Pierce, Rockford, IL) and transferred to nitrocellulose membranes. Anti-cyclooxygenase 1 antibody (ALEXIS Biochemicals, Lausen, Switzerland), anti-cyclooxygenase-2 antibody (BD Bioscience Pharmingen, San Diego, CA) and anti- β -actin (Sigma, St. Louis, MO) were used as a primary antibodies. Horseradish peroxidase-linked rabbit anti-mouse antibody was applied as a secondary antibody. Macrophage-IFN γ /LPS (BD

Bioscience Pharmingen) was used as a positive control for cyclooxygenase-2. The blots were analyzed using Super Signal assay (Pierce, Rockford, IL). β -actin served as a housekeeping protein. Cyclooxygenase-1 and cyclooxygenase-2 levels are expressed as ratios to β -actin protein levels.

Drugs

Krebs solution was prepared freshly and of the following composition (mM): 120.4 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 25.0 NaHCO₃, 1.2 NaH₂PO₄, 11.5 glucose; these chemicals were obtained from Merck (Darmstadt, Germany). The following drugs were used: Acetylcholine chloride, phenylephrine, indomethacine, sodium nitroprusside, N^G-Monomethyl-L-arginine acetate salt. Stock solution (10 mM) for indomethacine, was prepared in 96% ethanol. All other drugs were dissolved in deionized water. N^G-Monomethyl-L-arginine acetate salt was purchased from MP Biomedicals (Illkirch, France). All other compounds were purchased from Sigma. The concentrations presented in the concentration-curve responses are expressed as a final molar concentration in the organ baths.

Statistical analysis

Data are present as mean \pm SD and n refers to the number of animals in corresponding group. The contractile response to phenylephrine was expressed in mN and relaxant responses to acetylcholine and sodium nitroprusside are expressed as a percentage of the precontraction obtained with phenylephrine (0.1 mM). Concentration- response curves to phenylephrine and acetylcholine were characterized by Area Under the Curve, the maximal response (E_{\max}), and the negative logarithm of the molar concentration that caused half-maximal response (EC_{50}). Concentration-response curves of individual rings were plotted with SigmaPlot version 10.0 (Systat Software Inc, San Jose, CA). EC_{50} was calculated by Four Parameter Logistic Curve (SigmaPlot) for each ring. Statistical analysis was done with SPSS 16.0.2 for Windows (SPSS Inc., Chicago, IL). Differences between concentration-response curves were evaluated by Two-way Repeated-Measures ANOVA followed by Bonferroni test. Comparison of single parameters among multiple groups was done by One-Way ANOVA followed by Bonferroni test. Levene's test was used to confirm the homogeneity of variance. Independent sample t-test was used to compare the means of two groups where applicable. All tests were two-tailed and differences were considered significant at $P < 0.05$.

Results

Hemodynamic changes and routine lab parameters

To monitor the animals during the procedure, hemodynamic measurements and blood gas analyses were performed. After instrumentation at the start of the experimental protocol, hemodynamic parameters were comparable between the 3 experimental subgroups for a given type of anaesthesia. In mice undergoing blood withdrawal, shock was confirmed by a significant drop in blood pressure; MAP varied between 26 and 36 mmHg during the 90 min shock period followed by a near

normalization to baseline values in resuscitated animals (fig. 1). The main difference between both types of anesthesia was that in Sham isoflurane animals MAP was slightly lower (~ 10 mmHg throughout the protocol) compared to Sham isoflurane/nitrous oxide animals receiving adjunct nitrous oxide ($P<0.05$). Blood gases, $p\text{CO}_2$, Base excess, HCO_3^- , SaO_2 , Hb, and Hct did not significantly differ between both Sham groups at all time points measured (table 1). The pH was increased in Shock isoflurane group compared to Sham isoflurane. Adjunct nitrous oxide resulted in a similar pH in Sham isoflurane/nitrous oxide and Shock isoflurane/nitrous oxide groups (table 1). During shock, $p\text{O}_2$ was slightly increased in animals anesthetised with isoflurane only as compared to group with adjunct nitrous oxide (table 1). Other blood gas parameters were similar in both Shock groups.

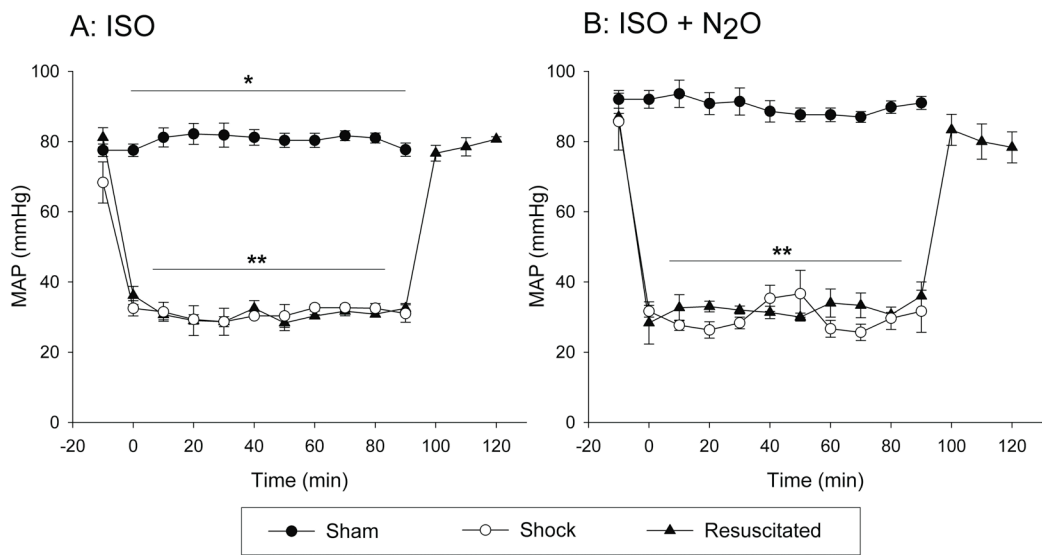


Figure 1. Mean arterial pressure (MAP, femoral artery) of Sham, Shock and Resuscitated groups during the experimental procedure in animals anesthetized with isoflurane (ISO, panel A), or anesthesia with isoflurane and adjunct nitrous oxide (ISO + N₂O, panel B). * denotes significantly different curves between Sham ISO and Sham ISO+N₂O ($P<0.05$). ** $P<0.05$ Sham vs corresponding Shock and Resuscitated groups

Table 1. Arterial blood gas data at the different time points of the experimental protocol

Parameters		Isoflurane			Isoflurane+Nitrous oxide		
		SHAM group	Shock group	Resuscitated group	SHAM group	Shock group	Resuscitated group
Number of animals weight, g		5	6	5	5	5	5
	0 min	26.9±2.5	27.7±3.3	26.2±1.7	26.6±3.0	28.9±0.9	29.0±3.2
	90 min	7.34±0.01 ^s	7.32±0.03	7.29±0.04	7.35±0.05	7.37±0.08	7.34±0.05
pH	0 min		7.42±0.03*			7.33±0.01	
	24 h			7.40±0.06			7.37±0.07
	90 min			4.9±0.4		3.9±0.4	4.6±0.5
pCO ₂ , kPa	0 min	3.5±0.8	4.5±0.6		3.4±0.1	3.4±0.9	
	24 h			2.8±0.4			3.5±0.1
	90 min		30.3±1.4*	31.9±0.9 [#]		25.3±1.7	25.2±1.9
pO ₂ , kPa	0 min	33.1±0.6	27.8±1.7		22.7±9.2	30.3±3.3	
	24 h			27.3±5.0			39.2±13.7
	90 min		-8.3±1.2	-8.3±1.6		-7.3±3.8	-6.6±2.2
BE, mmol/l	0 min	-10.5±2.4	-9.4±2.2		-10.3±2.5	-11.5±4.2	
	24 h			-11.9±2.4			-9.5±3.0
	90 min		16.6±1.4	17.3±0.1		16.4±2.7	18.0±1.5
HCO ₃ , mmol/l	0 min	13.6±2.7	13.8±2.6		13.6±1.6	13.0±3.9	
	24 h			12.1±1.7			14.6±2.1
	90 min		100.0±0	100.0±0		99.9±0.2	100.0±0
SaO ₂ , %	0 min	100.0±0	100.0±0		99.7±0.5	100.0±0	
	24 h			99.9±0.1			100.0±0
	90 min		7.9±0.4	7.9±0.4		8.4±0.3	8.6±0.2
Hb, mmol/l	0 min	6.9±0.9	4.6±0.6		7.3±0.6	5.6±1.3	
	24 h			3.1±0.3			3.6±0.4
	90 min		39.4±2.1	39.5±1.8		41.3±1.5	42.4±1.1
Hct	0 min	34.7±4.5	23.0±2.6		35.9±2.5	28.1±6.4	
	24 h			15.9±1.3			18.4±2.1

Data on the value of pH, pCO₂, pO₂, BE, HCO₃⁻, SaO₂, Hb, Hct in groups of mice anesthetized with isoflurane sole and isoflurane with adjunct Nitrous oxide. pCO₂, the partial arterial pressure of carbon dioxide; pO₂, the partial arterial pressure of oxygen; HCO₃⁻ bicarbonate; BE, Base excess; Hb, Hemoglobin; Hct, hematocrit; SaO₂, the oxygen saturation. * - P<0.05 Shock Isoflurane vs Shock Isoflurane+Nitrous oxide, # - P<0.05 Sham Isoflurane vs Shock Isoflurane, # - P<0.05 Resuscitated Isoflurane vs Resuscitated Isoflurane+Nitrous oxide

Table 2. The width of aorta rings in all groups

Groups	Isoflurane	Isoflurane/Nitrous oxide
Sham	1.8 ± 0.3	1.7 ± 0.1
Shock	1.7 ± 0.2	1.8 ± 0.2
Resuscitated	1.7 ± 0.1	1.7 ± 0.1

All data are presented as mean ± SD in mm.

Table 3. Parameters of Phenylephrine-mediated Contraction during Different Phases of Experimental Shock after Anaesthesia with either Isoflurane or Isoflurane with Adjunct Nitrous Oxide

Groups	Isoflurane			Isoflurane/Nitrous oxide		
	-logEC ₅₀	AUC	E _{max} , mN	-logEC ₅₀	AUC	E _{max} , mN
Sham	-6.9 ± 0.2	2.1 ± 1.6	1.5 ± 0.41	-7.0 ± 0.1	4.2 ± 1.0*	2.19 ± 0.31
Shock	-7.0 ± 0.3	7.8 ± 2.8†	3.24 ± 0.43†	-6.9 ± 0.2	6.2 ± 1.8	3.17 ± 0.44
Resuscitated	-7.0 ± 0.4	4.9 ± 1.8	2.28 ± 0.54	-6.8 ± 0.3	3.8 ± 1.2	2.1 ± 0.41

All data are presented as mean ± SD; AUC=area under the curve; E_{max}=maximal contractile response; ISO=isoflurane; N₂O=nitrous oxide. * P=0.048, Sham-ISO vs Sham-ISO/N₂O, two-tailed t-test; † P<0.05, Sham-ISO vs Shock-ISO, one-way ANOVA followed by Bonferroni test.

Contractile reactivity

Experiments *in vitro* were performed on freshly isolated aorta rings of similar length ($P=0.1$) in all groups (table 2). Contractile responses of isolated aortic rings were obtained by constructing concentration-response curves to phenylephrine. The contractile response to phenylephrine in the Sham isoflurane/nitrous oxide group was enhanced ($P < 0.05$) as compared with the Sham isoflurane group (fig. 2). This enhancement was characterized by an increased E_{\max} ($P < 0.05$) and an unchanged EC_{50} ($P=0.2$, table 3). The contractile response to phenylephrine in the Shock isoflurane and Resuscitated isoflurane groups was also enhanced as compared with the Sham isoflurane group ($P < 0.05$; fig. 2A). This enhancement was also characterized by an increased E_{\max} ($P < 0.05$) and an unchanged EC_{50} ($P=0.7$, table 3). However, the contractile response to phenylephrine was identical between the Shock isoflurane and Shock isoflurane/nitrous oxide groups and between Resuscitated isoflurane and Resuscitated isoflurane/nitrous oxide groups (fig. 2); i.e. no significant differences were observed in the E_{\max} and EC_{50} values between the corresponding two groups (table 3). These findings suggest that both shock and nitrous oxide increase contractility in a nonadditive manner.

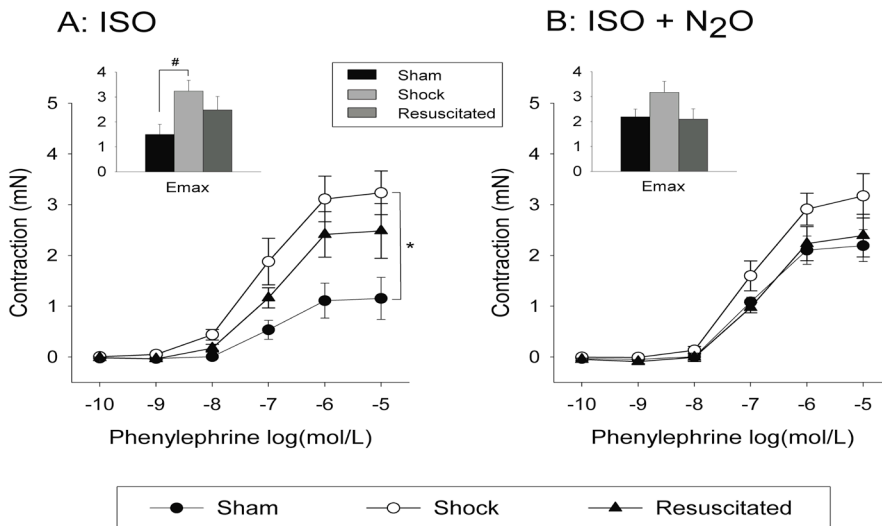


Figure 2. Contractile reactivity of aortic rings to phenylephrine of mice anesthetized with isoflurane (panel A) and isoflurane with adjunct nitrous oxide (panel B) in Sham, Shock and Resuscitated groups. Inserts show the responsiveness to phenylephrine presented as maximal response (E_{\max}) in mN. ISO=isoflurane; N₂O= Nitrous oxide. # $P<0.05$ Sham vs Shock Isoflurane. * $P<0.05$ Sham vs Shock Isoflurane

Relaxation responses to acetylcholine

Administration of acetylcholine to aortic rings from mice subjected to sham conditions caused a biphasic response, characterized by a relaxation, which was

maximal at 0.3 μ M, followed by a contractile response at concentrations ≥ 0.3 μ M (Sham groups in fig. 3, A and B). In Shock isoflurane and Resuscitated isoflurane mice, the contractile phase and hence the biphasic pattern in the concentration-response curve was fully suppressed (fig. 3A). In contrast, adjunct nitrous oxide preserved the biphasic pattern also after conditions of shock and resuscitation (fig. 3B). The latter is also reflected by the profound increase in acetylcholine concentration at which maximal relaxation was seen in Shock and Resuscitation groups that received anesthesia with isoflurane only (at 10 μ M), as compared to groups with adjunct nitrous oxide (at 1 μ M).

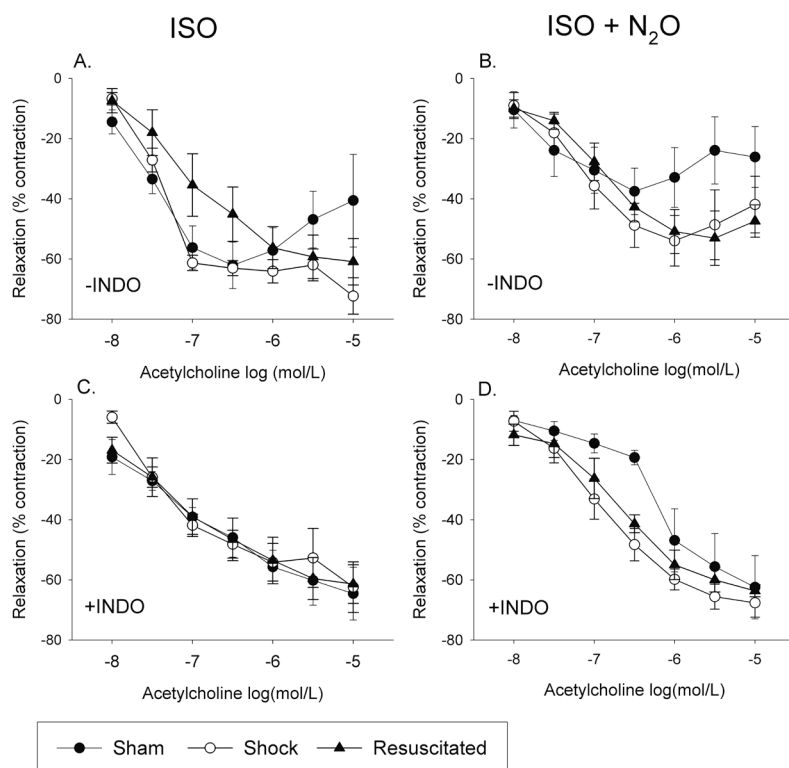


Figure 3. Acetylcholine induced relaxation of aortic rings from Sham, Shock and Resuscitated groups anesthetized with isoflurane (A and C) or isoflurane with adjunct nitrous oxide (B and D), and in the absence of indomethacin (A and B) or presence of indomethacin (C and D). Concentration-response curves of Sham groups (*closed circle*), Shock groups (*open circles*) and Resuscitated groups (*triangular down*) are expressed as percentage of preconstriction value to phenylephrine (0.1mM) and are present as mean \pm SD. INDO=indomethacin; ISO= isoflurane; N₂O= nitrous oxide.

The biphasic pattern in the acetylcholine concentration-response curve was also abolished in the presence of indomethacin, an inhibitor of cyclooxygenase (fig. 3, C and D). Consequently, under these conditions concentration-response curves for acetylcholine did not differ between groups. These findings imply the involvement of contractile prostaglandins in the upward stroke at higher concentrations of acetylcholine. Collectively, these findings indicate that the biphasic pattern of the

response to acetylcholine after conditions of shock and resuscitation was maintained by adjunct nitrous oxide.

While groups differed in anesthetic regimes and the levels of phenylephrine-precontraction, the relaxant response to sodium nitroprusside was similar in all groups (table 4), suggesting that acetylcholine responses represent changes in endothelial cell function rather than altered vascular smooth muscle response to nitric oxide.

Table 4. Maximal relaxant response to sodium nitroprusside in all groups

Maximal relaxation to SNP (%)					
Isoflurane			Isoflurane/Nitrous oxide		
Sham	Shock	Resuscitated	Sham	Shock	Resuscitated
101.2±21.7	99.4±8.1	101.5±1.8	101.2±1.3	103.4±7.1	98.0±11.3

Data are expressed as a percentage of phenylephrine-mediated precontraction and were calculated from concentration response curves at 0.1 mM of sodium nitroprusside. No differences were found between groups (one-way ANOVA). SNP, sodium nitroprusside.

Vascular cyclooxygenase-1 and cyclooxygenase-2 expression

Since short-term hemorrhagic shock differentially affected relaxation responses to acetylcholine with both modes of anesthesia, we subsequently investigated vascular protein expression of cyclooxygenase-1 and cyclooxygenase-2 in segments collected from the unstimulated abdominal aorta. In mice anaesthetized with isoflurane only, vascular cyclooxygenase-1 expression decreased significantly after shock, followed by a recovery during resuscitation (fig. 4, A,B and C). In contrast, cyclooxygenase-1 expression was preserved in groups receiving adjunct nitrous oxide. A similar pattern was observed for vascular cyclooxygenase-2 expression, but changes did not reach statistical significance (fig. 4, A and B). Collectively, these findings suggest that adjunct nitrous oxide mitigates the shock-induced decrease in the expression of cyclooxygenases, particularly cyclooxygenase-1.

Discussion

We investigated the effects of nitrous oxide adjunct to anesthesia with isoflurane on vascular reactivity in an experimental model of shock in mice. Our data show that hemorrhagic shock in animals that received isoflurane during the procedure induced profound changes in the subsequent vascular reactivity of isolated thoracic rings, consisting of an increased contraction to phenylephrine and a loss of cyclooxygenase mediated contraction to acetylcholine. These functional changes were accompanied by a decrease in the vascular expression of cyclooxygenase-1. Secondly, adjunct nitrous oxide augmented phenylephrine-induced contractility of Sham mice, but no further increase was observed when these animals underwent shock. In addition, adjunct nitrous oxide protected from the loss of contractile response to acetylcholine during shock and preserved the vascular expression of cyclooxygenase-1. Collectively, these findings suggest that vascular reactivity during different phases of shock may be preserved when adjunct nitrous oxide is employed,

which may involve (amongst others) a preservation of vascular cyclooxygenase-expression.

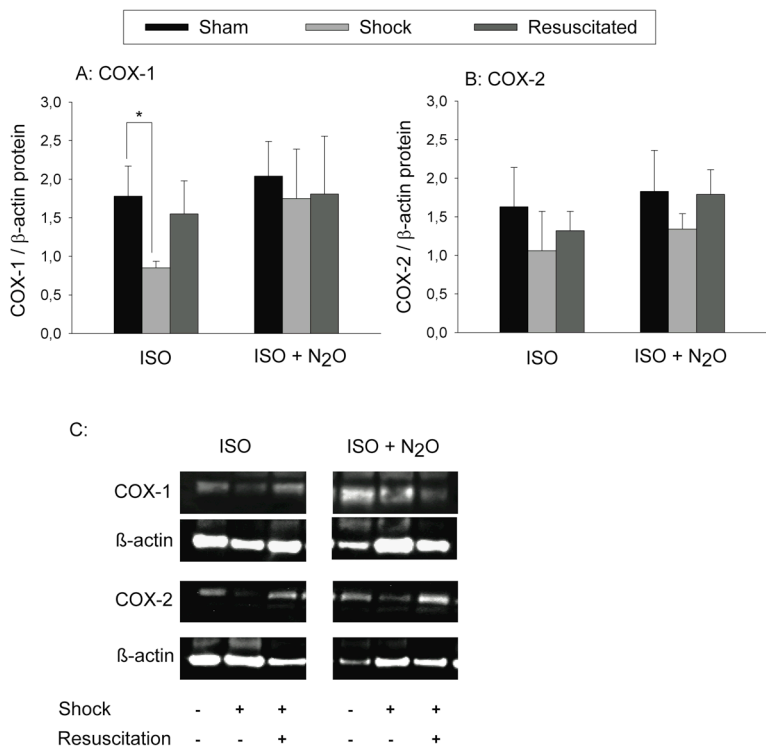


Figure 4. Expressions level of COX enzymes in aortic tissue from Sham, Shock and Resuscitated groups in groups anesthetized with isoflurane (ISO), or anesthesia with isoflurane and adjunct nitrous oxide (ISO+N₂O). Expression of COX-1 (A) and COX-2 (B) was assessed in Sham (n=5), Shock (n=5) and Resuscitated (n=5) groups. Data are normalized to the expression level of β -actin and presented in arbitrary units. Representative expressions pattern in Sham (n=5), Shock (n=5), Resuscitated (n=5) groups anesthetized with sole isoflurane with isoflurane and adjunct nitrous oxide (C). The difference between groups was borderline significant with One-Way ANOVA (P=0.06). However, independent t-test showed a significant reduction of COX-1 expression in Shock ISO versus Sham ISO (*P<0.05).

In this study, we investigated the effect of different anesthetic techniques applied during hemorrhagic shock *in vivo* on the vascular properties of aortic tissue that was harvested subsequently and examined in the absence of anesthetics. To our knowledge, the influence of anesthetics on vascular reactivity has not been explored in this way previously. Importantly, the changes observed in vascular reactivity in the shock group under isoflurane anesthesia imply that this procedure induces protracted changes in the vascular bed. Indeed, such view is in keeping with the observed downregulation of cyclooxygenase-1 in this group.

Systemic hypotension after isoflurane anesthesia has been reported previously,^{4, 12, 17} and its general depressant effects on hemodynamics are quite

notorious indeed.¹⁰ It may represent an important unwanted side effect of anesthesia, particularly during conditions of shock in which tissue perfusion already is at threat. Investigation of the underlying mechanisms was not within the scope of this study, but previous studies reported the involvement of decrements in processes such as myofilament calcium-sensitivity, intracellular calcium concentration, or voltage-gated calcium influx,¹⁸⁻²⁴ all of which might have contributed to decreased contractility after anesthesia. In the present study, decreased arterial pressure was observed together with decreased constriction to phenylephrine in isolated aortic ring preparation obtained from Sham mice anaesthetized with isoflurane, as compared to those receiving adjunct nitrous oxide. Recently, Pypendop et al showed that addition of 70% nitrous oxide to isoflurane anesthesia results in improved arterial and central venous pressures.¹² In addition, a study in patients under sevoflurane anesthesia suggests that adjunct nitrous oxide normalizes hemodynamic parameters of regional and systemic flow.¹¹ These findings suggest that hemodynamics may be better preserved during adjunct anesthesia with nitrous oxide compared to monoanesthesia with isoflurane. It should also be noted, however, that shock increased constriction to phenylephrine. It thus appears that contractile mechanisms become mobilized to maintain perfusion pressure during shock and overrule the effect of anesthesia. In keeping with that, contractility to phenylephrine was not further increased after shock with adjunct nitrous oxide, indicating that the effect of nitrous oxide was not additive.

Vascular responsiveness was also investigated by studying endothelial-mediated responses. As reported previously,²⁵ administration of acetylcholine caused relaxation at low concentrations and contraction at higher concentrations thus generating a biphasic concentration-response curve. Preincubation with indomethacine fully abolished the normal upward stroke in the concentration-response curve, thus indicating the involvement of contractile prostaglandins derived from cyclooxygenase herein. Interestingly, the upward stroke in the concentration-response curve was also lost in aorta preparations of shock-mice anesthetized with isoflurane, but not in those receiving adjunct nitrous oxide. Such findings suggest that shock may alter the production of contractile prostaglandins, which may be preserved during anesthesia with adjunct nitrous oxide. In mouse aorta, endothelial denudation abolishes the acetylcholine-mediated contractile responses,²⁵ implicating that contractile cyclooxygenases metabolites are derived from endothelial cells. In turn, this would implicate that the decreased cyclooxygenase-1 expression as observed following shock is caused by downregulation of the enzyme in endothelial cells. Previous studies suggest both isoforms of cyclooxygenase to be expressed in aorta endothelial and/or vascular smooth muscle cells,^{26, 27} and that both cyclooxygenase-1 and cyclooxygenase-2 may be involved in production of contractile prostaglandins. At present, there are few data on the influence of isoflurane on cyclooxygenase-protein expression. It has been reported that isoflurane produces cyclooxygenase-2 mediated anesthetic preconditioning, but it did not affect cyclooxygenase-1 and cyclooxygenase-2 protein expression.^{28, 29} Taken together, our results indicate that the loss of acetylcholine-induced contractile function in Shock-mice anesthetized solely with isoflurane, is due to decreased endothelial production of contractile prostaglandins

caused by downregulation of endothelial cyclooxygenase-1. Moreover, adjunct nitrous oxide counteracts these changes most likely due to a preservation of normal endothelial function.

Alternatively, nitrous oxide-evoked hyperhomocysteinemia may offer an explanation for enhanced vascular reactivity to phenylephrine in Sham animals, because of an increase in asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase.³⁰ Asymmetric dimethylarginine has been reported to increase arteriolar basal tone³¹ and participate in the maintenance of vasospasm.³² Previously, nitrous oxide anesthesia has been shown to enhance homocysteine concentration and impairment of endothelial function.³³ However, acetylcholine evoked relaxation of groups with and without nitrous oxide was similar in the presence of indomethacine, i.e. upon blockade of prostaglandin synthesis. Consequently, nitric oxide mediated relaxation does not differ between both groups, making the involvement of hyperhomocysteinemia unlikely.

Furthermore, nitrous oxide was reported to stimulate the sympathetic nervous system, which induces systemic and local vasoconstriction. This effect possibly explains the increased MAP during anesthesia with nitrous oxide.³⁴ Whether this phenomenon also explains the normalization of phenylephrine-mediated contraction in resuscitated animals from the nitrous oxide group remains to be established.

Aortic rings were studied in the absence of anesthetics in the organ bath. Our study clearly demonstrates vasomotor changes and decreased expression of cyclooxygenase-1 in the post-shock period, which were prevented by the use of nitrous oxide during the shock period. In view of the absence of volatile anesthetics and nitrous oxide in the organ baths, these results likely reflect vascular changes present in the immediate post-shock period, e.g. at the post anesthesia care unit or intensive care unit. However, it is unknown how these results obtained in isolated arteries correspond to vasoreactivity in *in vivo* conditions. Further, isoflurane and isoflurane/nitrous oxide groups differed in depth of anesthesia, since equivalent concentrations of isoflurane were used. However, as the study aimed to evaluate the effect of adjunct nitrous oxide, we chose to use the same isoflurane concentration in all experimental groups. Also, because the blood volume withdrawn for the induction of hemorrhagic shock was not assessed, we cannot comment on possible intra-operative differences in circulating volume. Finally, as the number of experiments was about six, small differences in calculated parameters of acetylcholine and phenylephrine-mediated responsiveness, or of cyclooxygenase expression may have been unnoticed.

Nowadays, despite intensive scientific discussion on the influence of adjunct nitrous oxide to volatile anesthesia,³³⁻³⁸ only few experimental data regarding this question are available. The present study shows positive effects of adjunct nitrous oxide on vasoresponsiveness after short-term hemorrhagic shock in mice, since this anesthetic agent normalized vascular reactivity after as observed under isoflurane anesthesia.

In summary, the findings of this study show that vascular reactivity after hemorrhagic shock is affected by the choice of general anesthesia. Short-term hemorrhagic shock under isoflurane anesthesia increased phenylephrine-mediated

contractile response and suppressed the acetylcholine-evoked contractile effect in thoracic mouse aorta, while adjunct nitrous oxide may attenuate changes in vascular reactivity caused by hemorrhagic shock and/or subsequent resuscitation. The results of this study also indicate that cyclooxygenase proteins participate in shock-dependent changes of vasoresponsiveness to acetylcholine. Our data suggest that the choice of the anesthetic regimen during emergency surgery for hemorrhagic shock may influence post-surgery vascular reactivity.

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